SYNTHESIS OF STATINS

The process for the synthesis of statins featuring the use of an early intermediate (4/?,6S)-6-(dialkoxymethyl)tetrahydro-2//-pyran-2,4-diol which already possesses the desired stereochemistry corresponding to the final statin.
SYNTHESIS OF STATINS

Field of the Invention

The present invention relates in general to the field of chemical technology and in particular to a process for the preparation of HMG-CoA reductase inhibitors, known also as statins, particularly to rosvuastatin. Specifically this invention relates to a preparation of a general but unique intermediate which can be used for the preparation of all statins.

Background of the Invention

Statins, of which the representative examples may be selected from rosvuastatin, cerivastatin, atorvastatin, fluvasstatin, pitavastatin, bervastatin, dalvastatin or their analogs or pravastatin, simvastatin, lovastatin or their analogs share a characteristic structure, consisting of respectively a heptenoic or heptanoic acid moiety (free acid, salt or lactone) connected to the aromatic or alicyclic core. Biological activity of statins is closely related to their stereochemistry, especially configuration at the chiral atoms of said heptenoic or heptanoic acid moiety.

Document US 5,527,916 describes a process for the separation of at least one isomer from a mixture of isomers of a tetrahydropyran-2-one having at least two chiral centers.

WO 2005/092867 A2 discloses the use of different lactone derivatives in the preparation of statins, particularly rosvuastatin.


An asymmetric aldol reaction with three differently substituted acetaldehyde substrates catalyzed by 2-deoxy-ribose-5-phosphate aldolase (DERA) shows that not all acetaldehyde derivatives are equally accepted as substrates for DERA (J. Am. Chem. Soc. 116 (1994), p. 8422-8423). The reaction time of the enzymatic catalysis was 6 days. The resulting lactone was oxidized with Br₂ and BaCO₃.

In WO 2006/1 34482 A1, a 2-deoxyribose-5-phosphate aldolase (DERA) catalyzed aldol addition step is included in a process for forming atorvastatin. The aldolase belongs to the class EC 4.1.2.4.
WO 05/1 18794 deals with an improvement of the DERA enzyme. The isolated mutant enzymes may be used for the preparation of a 2,4-dideoxyhexose or a 2,4,6-trideoxyhexose having a high variety of substituents.

The object of the present invention is to provide valuable intermediate compounds and processes as building blocks for effectively producing statins.

A further object of the invention is to produce intermediate compounds with inexpensive starting materials and simple equipment.

Disclosure of the Invention

The object is solved by providing (4fl,6S)-4-hydroxy-6-(dialkylxoxymethyl)-tetrahydro-2H-pyran-2-ol, which is protected or not protected at the 4-hydroxy position. This compound can be prepared by two different processes, either by a synthetic process or by an enzymatic process. The relevant synthetic process requires more synthetic steps compared to the enzymatic process however it was utilized to prepare a standard compound which confirmed the structure of the enzymatic product.

The significant enzymatic process allows a one step synthesis with relatively short reaction times and a high yield, and the product has a high stereochemical purity concerning diastereoisomeric excess. Further, according to the present invention, the above mentioned pyran-derivative can be produced from inexpensive starting materials and simple equipment.

One aspect of the invention is a process for preparing a compound of formula IV or V

\[
\begin{align*}
\text{IV} & \quad \text{or} \quad \text{V} \\
\end{align*}
\]

wherein \( R_1 \) is a protecting group which comprises the following steps:

a) converting a compound of formula I'.

\[
\begin{align*}
\end{align*}
\]
wherein $R_2$ and $R_3$ are independently selected from CrC$_4$-alkyl or together form a cyclic structure of formula (CH$_2$)$_n$ wherein $n$ is from 2 to 6,

into a compound of formula II

![Diagram](image)

wherein $R_2$ and $R_3$ are defined as above;

b) subsequently converting said compound obtained in step (a) into a compound of formula III

![Diagram](image)

wherein $R_1$ is a protecting group, $R_2$ and $R_3$ are defined as above, and;

c) subsequently cleaving the acetal-compound of formula III obtained in step (b). This process provides a short and efficient 3-step synthesis to arrive at compounds of formula IV or V.

Preferably the substrate of step (a) is selected from a compound of formula I', wherein $R_2$ and $R_3$ are methyl. The reactants for the oxidation step (a) should be inexpensive and afford a high yield. Thus the oxidation is performed preferably with Br$_2$ and BaCO$_3$.

Step (b) is preferably performed by introducing $R_1$ being a silyl protecting group. Furthermore it is preferred to perform step (b) with t/t-butylmethylysilyl chloride.

Preferably step (c) of the process is performed with I$_2$ and acetone. In particular, step (c) is performed in an anhydrous medium to provide the aldehyde compound of formula IV. However the hydrate form V is preferred for isolation and storage. Therefore furthermore it is preferred to perform step (c) in a solvent containing water to provide the hydrate compound of formula V.

Another aspect of the invention is a process for providing the compound of formula IV.
wherein R₁ is a protecting group,
characterized by providing the compound of formula V

wherein R₁ is a protecting group,
dissolved in a solvent selected from the group of aromatic hydrocarbons, aliphatic hydrocarbons, chlorinated aromatic or aliphatic hydrocarbons and aliphatic ethers.

In particular, the solvent of this process is selected from toluene, heptane, methylcyclohexane, cyclohexane, hexane, pentane, dichloromethane, chloroform, 2-chlorotoluene, 3-chlorotoluene, 4-chlorotoluene, 'BuMeO, Et₂O, Me₂O and THF. This group of solvents favours the provision of the aldehyde of formula IV in an equilibrium of the compounds of formula IV and V. Thus the hydrate form of formula V can be converted to the aldehyde of formula IV without being isolated, but by carrying out the reaction in said solvents.

A further aspect of the invention is a use of any of the compounds of formula I

wherein R₁ is H or protecting group; and
R₂ and R₃ are independently selected from CrC₄-alkyl for the synthesis of a compound of formula IV or V
Another aspect of the present invention is a compound of formula I, wherein $R_1$ is H or a protecting group; and $R_2$ and $R_3$ are independently selected from C$_1$-C$_4$-alkyl, or together form a cyclic structure of formula (CH$_2$)$_n$ wherein $n$ is from 2 to 6. The compound of formula I wherein $R_1$ is H or te/t-butyldimethylsilyl is preferred. In particular, $R_2$ and $R_3$ are methyl.

Another aspect of the present invention is a process for preparing a lactol compound of formula I', wherein $R_2$ and $R_3$ are independently selected from C$_1$-C$_4$-alkyl, or together form a cyclic structure of formula (CH$_2$)$_n$ wherein $n$ is from 2 to 6, which comprises the step of reacting a substrate of formula X.
wherein R_2 and R_3 are defined as above, with acetaldehyde under aldolase-catalysed aldotriose condensation conditions to form the corresponding lactol compound I'. Preferably this process comprises the additional steps of:

a) converting said lactol compound of formula I' into compound of formula IV

\[
\text{IV}
\]

b) subjecting said compound obtained in step (a) to conditions sufficient to produce a statin or a pharmaceutically acceptable salt thereof. Preferably the statin of step (b) is rosuvastatin. More preferably the acceptor substrate X is selected from the group consisting of: 2,2-dimethoxyethanal, 2,2-diethoxyethanal, 2,2-dipropoxyethanal, 2,2-dibutoxyethanal, 1,3-dioxolane-2-carbaldehyde, 4-methyl-1,3-dioxolane-2-carbaldehyde, 4-ethyl-1,3-dioxolane-2-carbaldehyde, 4-propyl-1,3-dioxolane-2-carbaldehyde, 4-butyl-1,3-dioxolane-2-carbaldehyde, 4,5-dimethyl-1,3-dioxolane-2-carbaldehyde. By using aldolase-catalysed aldotriose condensation conditions, the number of reaction steps to arrive at IV can be reduced.

It is preferred that the aldolase catalyzing the aldotriose condensation is 2-deoxyribose-5-phosphate aldolase (DERA). It may be useful to screen different types of DERA enzymes in order to find an enzyme having broader substrate specificity. Furthermore a DERA enzyme may be tailored for a specific substrate. For these reasons different mutant DERA enzymes may be tested.

More particularly said aldolase is selected from the group consisting of DERA 01, DERA 02, DERA 03, DERA 04, DERA 05, DERA 06, DERA 07, DERA 08, DERA 09, DERA 10, DERA 11, DERA 12, DERA 13, DERA 14, DERA 15, DERA 16, DERA 17, DERA 18, DERA 19, DERA 20, DERA 21, DERA 22, DERA 23 or an aldolase having an amino acid sequence identity of at least about 70 % to amino acid sequence of any of said aldolases. More particularly said aldolase is selected from the group consisting of an aldolase having an amino acid sequence identity of at least about 70 % to amino acid sequence of SEQ ID NO: 2, an aldolase having an amino acid sequence identity of at least 90 % to amino acid sequence of SEQ ID NO: 5 or an aldolase having an amino acid sequence identity of at least 90 % to amino acid sequence of SEQ ID NO: 17.

The process aspect of the invention can be effectively accomplished in reaction conditions wherein a buffer having no primary, secondary or tertiary amino group is used. Such a buffer
provides higher yields and less impurities, because it does not undergo chemical reactions with condensation reaction intermediates. In particular the buffer is a phosphate buffer. Preferably the pH value for the aldolase-catalysed aldol condensation is maintained in the range from 6 to 10. More preferably the pH is maintained with a buffer in the range of 7 to 9. In particular the process comprises the steps of

i) performing the aldolase-catalysed aldol condensation in an aqueous medium at a pH value of 7 to 9 at a temperature of 30 - 50 °C to form a reaction mixture,

ii) maintaining said reaction mixture at said temperature and pH value for a time period between 1 h and 6 h; and

iii) recovering said compound of formula I'.

A further aspect of the invention is a use of aldolase for the reaction of a substrate of the formula X

\[
\begin{align*}
R_2 & \quad O \\
R_3 & \quad O
\end{align*}
\]

wherein \(R_2\) and \(R_3\) are independently selected from d-C \(_4\)-alkyl, or together form a cyclic structure of formula \((\text{CH}_2)_n\) wherein \(n\) is from 2 to 6, with acetaldehyde under aldolase-catalysed aldol condensation conditions to form the corresponding lactol compound of the formula \(I'\)

\[
\begin{align*}
\text{HO} & \quad \text{(R)} \\
\text{(S)} & \quad \text{OH} \\
R_2 & \quad \text{O} \\
R_3 & \quad \text{OR_3}
\end{align*}
\]

wherein \(R_2\) and \(R_3\), wherein \(R_2\) and \(R_3\) are defined as above. In particular said aldolase is 2-deoxyribose-5-phosphate aldolase (DERA). More particularly aldolase is selected from the group consisting of DERA 01 to DERA 23 as described above. In particular said aldolase is comprised within a living whole cell, or is comprised within an inactivated whole cell, or is comprised within a homogenized whole cell, or is comprised within a cell free extract, or is a purified enzyme, or is immobilized, or is in a form of an extracelularly expressed protein.

Another aspect of the invention is a process for preparing a HMG CoA reductase inhibitor, characterized in that the compound of formula \(I'\)
wherein \( R_2 \) and \( R_3 \) are independently selected from \( \text{CrC}_4 \)-alkyl or together form a cyclic structure of formula \((\text{CH}_2)_n\) wherein \( n \) is from 2 to 6,

is provided as an intermediate compound to prepare said HMG CoA reductase inhibitor. Preferably the process comprises the following steps:

i) converting compound I' into a compound of formula IV

\[
\text{IV}
\]

wherein \( R_1 \) is a protecting group; and

ii) reacting said compound of formula IV under conditions sufficient to produce a HMG CoA reductase inhibitor or a pharmaceutically acceptable derivative thereof. More preferably the conditions of step (ii) are set by a Wittig coupling with an appropriate phosphonium salt to give rosuvastatin or a derivative thereof, preferably wherein the process comprises the steps of:

Na) providing a phosphonium salt having the formula VI

\[
\text{VI}
\]

wherein \( R_x, R_y, \) and \( R_z \), are the same or different and are selected from optionally substituted \( \text{C}_8 \)-alkyl or \( \text{C}_3-\text{C}_6 \)-cycloalkyl or \( \text{Cl}-\text{Cs-alkenyl} \) or \( \text{C}_5-\text{Cs-cycloalkenyl} \) or ary1,

and \( Y \) is an anion, preferably halogen or \( \text{RCOO}^- \) anion, more preferably chloride, bromide or trifluoroacetate;

to give a compound of formula VII
and lib) subsequently converting the compound VII to rosuvastatin or a salt thereof.

In this process, it is preferred that $R_2$ and $R_3$ are methyl.

Yet another aspect of the invention is a process for preparing a HMG CoA reductase inhibitor, wherein the compound of formula II

wherein $R_2$ and $R_3$ are independently selected from $\text{C}_4$-alkyl or together form a cyclic structure of formula $(\text{CH}_2)_n$ wherein $n$ is from 2 to 6 is provided as an intermediate compound to prepare said HMG CoA reductase inhibitor, which process comprises the steps of:

A) converting a compound of formula II into a compound of formula IV

wherein $R_1$ is a protecting group; and

B) reacting said compound of formula IV by a Wittig coupling with an appropriate phosphonium salt to give a HMG CoA reductase inhibitor. Preferably the HMG CoA reductase inhibitor is rosuvastatin or a derivative thereof. Furthermore, it is preferred that $R_2$ and $R_3$ are methyl. Rosuvastatin or a derivative thereof includes a free acid form, a salt form such as sodium, potassium or especially calcium salt, and e.g. acid esters or lactone rings, but is not limited
Detailed description of the Invention

5 The present invention relates in general to the synthesis of HMG CoA reductase inhibitors (statins) wherein the compound of formula IV

![IV](image)

wherein R₁ is a protecting group,

10 is reacted with an appropriate phosphonium salt, phosphinoxide or phosphonate of the heterocyclic or alicyclic skeleton of a statin, such as a compound of formula VI

![VI](image)

wherein Y is a suitable anion.

15 The compound IV (in particular IV wherein R₁ is tert-butyldimethylsilyl) is prepared from an intermediate I' (R₂ and R₃ are independently selected from CrC₄-alkyl, or together form a cyclic structure of formula (CH₂)ₙ wherein n is from 2 to 6) in accordance with the following general scheme:

![Scheme](image)

or specific for I" wherein R₂ and R₃ are both Me, shown in the following scheme:
Furthermore the present invention provides for an enzymatic synthesis of intermediate I', which is chemically \((4R,6S)-6\text{-}(dialkoxymethyl)\text{tetrahydro-2H-pyran-2,4-diol}\) or \((4S,6S)-6\text{-}(\text{alkylenedioxy})\text{tetrahydro-2H-pyran-2,4-diol}\) of a general formula I'.

\[
\text{R}_2\text{O} \quad \text{R}_3
\]

wherein \(\text{R}_2\) and \(\text{R}_3\) are independently selected from \(\text{d-C}_4\)-alkyl, or together form (an optionally \(\text{CrC}_4\)-alkyl substituted) cyclic structure of formula \((\text{CH}_2)_n\) wherein \(n\) is from 2 to 6.

The protecting group \(\text{Ri}\) of the present invention may be any conventionally used protecting group, in particular alkyl, acyl, silyl or similar group, more particularly selected from acetyl (Ac), pivaloyl (Piv), p-toluenesulfonyl (TOS), \(\beta\)-methoxyethoxymethyl ether (MEM), methoxymethyl ether (MOM), p-methoxybenzyl ether (PMB), methylthiomethyl ether, \(f\)-butyl, tetrahydropyranyl (THP), benzyl (Bn), diphenylmethyld or triphenylmethyld group, preferably silyl protecting group which can be represented by a formula \(\text{SiR}_1\text{R}_2\text{R}_3\) in which \(\text{R}_1, \text{R}_2, \text{R}_3\) are independently selected from alkyl (preferably \(\text{C}_1\text{-C}_6\)) or aryl (preferably \(\text{C}_5\text{-C}_{10}\)), such as \(\text{SiMe}_3\) (TMS), \(\text{SiMe}_2\text{Bu}\) (TBDMS), \(\text{Si(Z-Pr)}_3\) (TIPS), \(\text{SiPh}_2\text{Bu}\), \(\text{SiMe}_2\text{Ph}\).

The feature of an early intermediate I', which is chemically \((4R,6S)-6\text{-}(dialkoxymethyl)\text{-tetrahydro-2H-pyran-2,4-diol}\) or \((4S,6S)-6\text{-}(\text{alkylenedioxy})\text{tetrahydro-2H-pyran-2,4-diol}\) and in particular I", which is chemically \((4R,6S)-6\text{-}(\text{dimethoxymethyl})\text{-tetrahydro-2H-pyran-2,4-diol}\), is that it possesses the desired stereochemistry, avoiding subsequent separations of later intermediates. On the other hand I' or I" is chemically aldehyde acetal which behaves as a masked (protected) aldehyde which eliminates the necessity for the oxidation step in the synthesis of statins.
Compounds IV and IV, or hydrate thereof, obtained from I" or I' can be further used to prepare rosuvastatin as outlined on the following scheme:

To produce other statins, the (2S,4fl)-4-(protected)-6-oxo-tetrahydro-2/-/-pyran-2-carbaldehyde IV or its hydrate V should be reacted under the condition of a Wittig coupling with an appropriate reagent followed by hydrogenation when needed.

The appropriate reagent is a heterocyclic or alicyclic derivative (skeleton of statin) of a following formula:

wherein A can be a bond or O;

and wherein R_x, R_y, and R_z, are the same or different and are selected from optionally substituted C^-Cs-alkyl or C_3^-C_6-cycloalkyl or d-C_6-alkenyl or C_5^-C_6-cycloalkenyl or aryl;

and Y is an anion, preferably halogen or RCOO^- anion, more preferably chloride, bromide or trifluoroacetate;
and Het is selected so that it forms a heterocyclic or alicyclic skeleton of a statin; other HMG-CoA reductase inhibitors (preferably selected among cerivastatin, fluvastatin, pitavastatin, bervastatin, dalvastatin) can be analogously prepared.

The heterocyclic or alicyclic skeleton (Het) of statins is in particular selected from:

![Chemical structures]

Compounds IV or IV may in the presence of water, if in a liquid state or dissolved in an organic solvent, exist in an equilibrium with its hydrated form V (or V)

![Chemical structure V]

and may be isolated in an aldehyde or a hydrate form. The aldehyde form can be isolated from anhydrous media by evaporation while the hydrate form can be isolated from solvents containing water by evaporation or precipitation and filtration. The hydrate form is preferable for isolation and storage. The Witting reaction proceeds with the aldehyde form, however both forms can be used if the reaction is carried out in a solvent in which the aldehyde form is favoured in the equilibrium. In chlorinated hydrocarbons (such as chloroform, dichloromethane), hydrocarbons (such as hexane and cyclohexane) and particularly in aromatic hydrocarbons (such as toluene or the chlorinated analogue thereof), the equilibrium is shifted completely towards the aldehyde form. The use of toluene as the solvent for the Wittig reaction (with phosphonium salt, phosphinoxide or phosphonate of heterocyclic or alicyclic skeleton of a statin) significantly increases the yield compared to the commonly used THF because in THF aldehyde and hydrate are present in approximately equal amounts while aromatic hydrocarbons favour the aldehyde.
In general, I’ can be converted to II’ ((4/3,6S)-6-(dimethoxymethyl)-4-hydroxytetrahydro-2/-/-pyran-2-one) or I’ to analogous II ((4/3,6S)-6-(dialkoxy methyl)-4-hydroxytetrahydro-2/-/-pyran-2-one) or ((4/3,6S)-6-(alkylenedioxymethyl)-4-hydroxytetrahydro-2/-/-pyran-2-one) by oxidation with a suitable oxidizing agent which can be selected from bromine, N-iodosuccinimide / tetra-n-butylammonium iodide in dichloromethane or NaOCl in an appropriate solvent, in particular with bromine in the presence of weak bases, in particular BaCO₃ which does not hydrolyze the formed lactone in a suitable solvent which can be polar and protic, such as water at temperatures from O to 40 °C. The reaction proceeds well if there is an excess of bromine compared to the substrate and excess of BaCO₃ compared to the bromine. The product is conveniently isolated by extraction from water with a water immiscible solvent, such as ethyl acetate, followed by flash chromatography.

To II’ or II, a suitable protecting group, which can be any conventionally used protecting group, in particular silyl protecting group, is introduced, in particular by a reaction with tetra-n-butyldimethylsilyl chloride. The reaction is conveniently done in the presence of a base, selected from amines, imidazoles and pyridines, preferably imidazole in solvents selected from amides: N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA), hexamethylphosphor triamide (HMPTA); N-methylpyrrolidone (NMP); N,N,N,N,N-v-dimethylpropyleneurea (DMPU); N,N,N,N,N/tetramethylurea (TMU); dimethylsulfoxide (DMSO); nitriles (acetonitrile), chlorinated hydrocarbons (dichloromethane, chloroform), aromatic hydrocarbons (toluene), preferably in DMF where starting material and reagents are well soluble. The reaction can be performed at temperatures between -10 °C to 30 °C. Preferably at O °C. The reaction is accomplished in a period from one hour up to a day, preferably in 12 to 24 hours. The product (I'M’ or III) can be isolated by evaporation of the chlorinated hydrocarbon solvent followed by flash chromatography or by dilution with water and extraction to a water immiscible solvent, such as ethyl acetate, followed by evaporation when amide solvent is used for the reaction.

I’M’ can be converted to IV (or III to IV) by a hydrolytic cleavage of acetals by acid catalysts which do not catalyse the cleavage of lactone by using Broensted or Lewis acids in an organic solvent, preferably Lewis acids, such as FeCl₃·6H₂O, FeCl₃·SiO₂, CuCl₂·2H₂O, Zn(NO₃)₂·6H₂O, (NH₄)₂Ce(NO₃)₆, CeCl₃·7H₂O, TiCl₄, ZnBr₂, SnCl₂·2H₂O, LiBF₄ in wet acetonitrile, most preferably ZnBr₂ in methylene chloride; by transacetalization reaction with ketones in the presence of catalysts preferably with aceton in the presence of iodine; by pyridinium p-toluenesulfonate in wet aceton; DDQ; Montmorillonite K₁₀; CBr₄/MeCN/H₂O; Me₃SiCl.

The hydrated form of formula V or V may be used for the Wittig reaction without further purification by dissolution in an appropriate solvent (toluene or dichloromethane) where
dehydration to the compound of formula IV or IV occurs. In the specific embodiment related to rosuvastatin, in the subsequent reaction step, (2S,4/?)-4-(fe/t-butyl(dimethyl)silyloxy)-6-oxo-tetrahydro-2H-pyran-2-carbaldehyde (IV), or IV if another protecting group is used, can be reacted under the conditions of a Wittig coupling (in the presence of a base) with a ((4-(4-fluorophenyl)-6-isopropyl-2-(N-methylmethylsulfonamido)pyrimidin-5-yl)methyl)triphenylphosphonium halide or any other ((4-(4-fluorophenyl)-6-isopropyl-2-(N-methylmethylsulfonamido)pyrimidin-5-yl)methyl)phosphonium salt or alternatively di-/propyl(4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonfyl)amino]-5-pyrimidinyl)methylphosphonate or any other ((4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonfyl)amino]-5-pyrimidinyl)methylphosphonate ester to give N-(5-(\((\text{E})-(2S,4/-)?\)-4-(fe/t-butyl(dimethyl)silyloxy)-6-oxo-tetrahydro-2-//-pyran-2-yl)vinyl)-4-(4-fluorophenyl)-6-isopropyl(pyrimidin-2-yl)- N'-methylmethanesulfonamide (VM), or an analogue VII if another protecting group is used. As a base, lithium hexamethyldisilazane (LiHMDS), potassium hexamethyldisilazane (KHMDS), sodium hexamethyldisilazane (NaHMDS), lithium diisopropylamide (LDA), sodium hydride, butyllithium or Grignard reagents, preferably sodium hexamethyldisilazane may be used. When the source of IV is the hydrate thereof form V or a mixture of IV and the hydrate form V thereof, which is dissolved in ethers selected from THF, Et₂O, Pr₂O, 'BuMeO; hydrocarbons selected from: pentane, hexane, cyclohexane, methylcyclohexane, heptane; aromatic hydrocarbons selected from toluene or the chlorinated derivatives thereof; chlorinated hydrocarbons selected from: chloroform and dichloromethane or in mixtures of those solvents, the water released from the hydrate should be removed prior to the addition to the formed ylide solution. The preferred solvents for the reaction are anhydrous toluene and dichloromethane. The reaction can be performed at temperatures between -80 °C and 90 °C preferably at Oto 90 °C, more preferably at 80 - 90 °C. The reaction is accomplished in 1 - 12 hours. Isolation of the crude product with extraction can be performed with AcOEt, ethers or alkanes as above. Preferably with 'BuMeO.

The protecting group may be removed and the lactone opened to produce a rosuvastatin free acid or a salt thereof, optionally an amine, which may be converted to hemicalcium salt. The deprotection can be performed at temperatures between 0 °C to 80 °C. Preferably at 20 or 40 °C in a suitable solvent, preferably a solvent selected from alcohols, acetic acid, THF, acetonitrile, methyltetrahydrofuran, dioxane, CH₂Cl₂, more preferably in alcohols and a mixture of THF/AcOH. The usual deprotecting reagents may be used, such as tetra-n-butylammonium fluoride, ammonium fluoride, AcCl, FeCl₃, TMSCl/HF-2H₂O, chloroethylchloroformate (CEC), Ph₃PCH₂COMeBr. The opening of the lactone preferably takes place in a 4:1 to 2:1 mixture of THF/H₂O as well as in pure THF at temperatures between 20 °C to 60 °C with a suitable alkali such as NaOH, KOH, ammonia or amines. The hydrolysis is accomplished in 30 minutes (at 60 °C) to 2 hours (at 20 °C). After the hydrolysis step, evaporation of THF can be conducted at
temperatures between 10 °C to 50 °C under the reduced pressure, and conversion to the
calcium salt, preferably by the addition of Ca(OAc)$_2$.xH$_2$O, which can be added in one portion or
dropwise in 5 to 60 minutes, can be performed at temperatures between 0 °C to 40 °C. After the
addition of Ca(OAc)$_2$.xH$_2$O, the resulting suspension can be stirred at temperatures between 0
°C to 40 °C from 30 minutes to 2 hours. To produce other statins, (2S,4R)-4-(tert-butyl)-
dimethylsilyloxy)-6-oxo-tetrahydro-2H-pyran-2-carbaldehyde (IV) (or the compound of the
general formula IV) should be reacted under analogous conditions of a Wittig coupling with an
appropriate reagent followed by hydrogenation when needed.

The standard compound of formula I’ was prepared by this chemical synthesis in order to
confirm the structure of the enzymatic product. Enzymatically prepared compound of formula I’
is chemically equivalent to the compound of formula I’ prepared by the chemical synthesis.

Compounds of formula I’

\[
\begin{align*}
\text{HO} & \\
\text{(R)} & \\
\text{(S)} & \\
\text{O} & \\
\text{R}_2 & \\
\text{O} & \\
\text{OR}_3 & 
\end{align*}
\]

wherein R$_2$ and R$_3$ are independently selected from d-C$_4$-alkyl, or together form a cyclic
structure of formula (CH$_2$)$_n$ wherein n is from 2 to 6,

can be prepared from (4/5,6S)-4-(profecfec)-6-(iodomethyl)-tetrahydropyran-2-one, such as
(4fl,6S)-4-(tert-butyl(dimethyl)silyloxy)-6-(iodomethyl)-tetrahydropyran-2-one (J. Chem. Soc,
Perkin Trans. 1(1991) 133-140) by an acetate substitution, subsequent reaction with [t-
Bu$_2$SnO(Cl)]$_2$ and the subsequent oxidation with Dess-Martin periodinane (DMP) followed by
the reaction with trialkyl ortoformate and reduction with di-s-butylaluminium hydride yielding
(4fl,6S)-4-(protected)-6-(dialkoxy)methyl)tetrahydro-2H-pyran-2-ol, from which the protecting
group is removed with tetra-n-butylammonium fluoride. This synthetic route for the compound of
the formula I’ is shown in the following scheme:
In another embodiment, compound I" may be prepared as outlined on the scheme below starting from dehydroacetic acid P1 which is hydrolysed with 90 % sulphuric acid at 130 °C, further oxidizing the obtained pyrone P2 by selenium dioxide in diglyme to give a formyl substituted pyrone P3. The aldehyde is further transformed to the corresponding dimethyl acetal P4 by the reaction with methanol in the presence of gaseous hydrogen chloride, then the pyrone ring is reduced by hydrogenation in a two step process first by using 10 % palladium on carbon to obtain a dihydro-derivative P5 and then Raney-nickel to obtain a mixture of fully saturated diastereoisomers of the structure P6. The mixture is resolved by transesterification with vinyl acetate in tetrahydrofuran in the presence of a lipase at 40 °C in which the desired A(R)-Q(S) isomer is not acetylated. Finally after removing the lipase by filtration and the solvent by evaporation, the A(R)-Q(S) isomer which is not acetylated is isolated from other acetylated diastereoisomers by column chromatography on silica gel by using ethyl acetate / dichloromethane mixtures. The obtained 6(S)-dimethoxy-4(/'¿)-hydroxytetrahydropyran-2-one II' is reduced by diisobutylaluminium hydride to obtain I'. 
In yet another embodiment, the invention provides an enzymatic process using a substrate of the formula X and acetaldehyde to form the corresponding lactole I' in an aldolase catalysed aldol condensation reaction as shown in the following scheme:

wherein R₂ and R₃ are independently selected from d-C₄-alkyl, or together form an optionally substituted cyclic structure of formula (CH₂)ₙ where n is from 2 to 6. Structure I' according to the present invention has a strictly defined stereoisomery at the positions 4 and 6, while other chiral centers may be present in both possibilities forming mixtures of epimers. The changeable chiral
centers are not essential for the synthesis of the final product of the invention as the chirality of those carbon atoms is lost during the synthetic procedure.

The term "aldolase-catalyzed aldol condensation conditions" as used herein refers to any aldol condensation conditions known in the art that can be catalyzed by an aldolase, as described herein. In particular, the aldolase-catalysed aldol condensation conditions are such that they allow forming and accumulation of the desired product. These conditions include in one aspect that the aldolase is an active enzyme provided at sufficient load to be able to perform the sequential condensation, in another aspect that the substrate and acetaldehyde are present in the reaction in an amount that does not inhibit the activity of the aldolase. Furthermore the conditions include in one aspect that the temperature, pH, the solvent composition, agitation and length of the reaction allow accumulation of the desired product, in another aspect that said conditions do not have detrimental effect on the product stability. Specifically those conditions are defined by values disclosed in the examples.

Aldolase activity towards the above substrate of the formula X means that the specified enzyme is either purified and/or isolated, or immobilized or within a living cell, or comprised within an inactivated whole cell, or comprised in a homogenized cell material, or in a cell free extract, which will catalyze the above reaction of the substrate X with acetaldehyde to form the corresponding lactol compound of the formula I'.

The term "conditions sufficient to produce statin (in particular rosvustatin) or a pharmacologically acceptable salt thereof" as used herein refers to those means described in the art, including those means described herein.

The term an "organism over expressing biologically active form of an aldolase" as used herein refers to any organism having the aldolase expression under control of a strong promoter, and wherein the aldolase is expressed at high levels (compared to w.t. expression control) and is accumulated intracellularly or extracellularly. The process of making such an organism is well known to a person skilled in the art. The present invention provides an example of making such an organism.

An aldolase for use in the present invention may be any compound that has aldolase activity towards the above substrate of formula X. In one embodiment of the invention, the aldolase is a 2-deoxyribose-5-phosphate aldolase (DERA). Examples of a suitable DERA - aldolase include, but are not limited to: DERA 01, DERA 02, DERA 03, DERA 04, DERA 05, DERA 06, DERA 07, DERA 08, DERA 09, DERA 10, DERA 11, DERA 12, DERA 13, DERA 14, DERA 15, DERA 16,
DERA 17, DERA 18, DERA 19, DERA 20, DERA 21, DERA 22 and DERA 23 which are identified by their nucleotide sequences or respective codon optimized nucleotide sequences or amino acid sequences set forth in the sequence listings.

In general, any of the DERA aldolases known in the art may be used for the reaction regardless of their sequence identity to the above listed DERA aldolases. The invention provides examples of performing said reactions successfully with two different aldolases having only 30.1% identity. The yields of the reaction however may depend on each aldolase substrate specificity and inhibitory effects of the substrates on each aldolase.

DERA 01 is an aldolase having a nucleotide sequence of SEQ ID NO: 1 or an amino acid sequence of SEQ ID NO: 2; DERA 01 (E. Coli) is commercially available from Sigma Aldrich, St. Louis, MO, USA, under the catalog number 91252.

DERA 02 is an aldolase having a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 4 or an amino acid sequence of SEQ ID NO: 5; DERA 02 is described in William A. Greenberg, et al., PNAS (2004), Vol. 101, No. 16, p. 5788.

DERA 03 is an aldolase having a nucleotide sequence of SEQ ID NO: 6 or an amino acid sequence of SEQ ID NO: 7

DERA 04 is an aldolase having a nucleotide sequence of SEQ ID NO: 8 or an amino acid sequence of SEQ ID NO: 9

DERA 05 is an aldolase having a nucleotide sequence of SEQ ID NO: 10 or an amino acid sequence of SEQ ID NO: 11

DERA 06 is an aldolase having a nucleotide sequence of SEQ ID NO: 12 or an amino acid sequence of SEQ ID NO: 13

DERA 07 is an aldolase having a nucleotide sequence of SEQ ID NO: 14 or an amino acid sequence of SEQ ID NO: 15

DERA 08 is an aldolase having a nucleotide sequence of SEQ ID NO: 16 or an amino acid sequence of SEQ ID NO: 17

DERA 09 is an aldolase having a nucleotide sequence of SEQ ID NO: 18 or an amino acid sequence of SEQ ID NO: 19

DERA 10 is an aldolase having a nucleotide sequence of SEQ ID NO: 20 or an amino acid sequence of SEQ ID NO: 21

DERA 11 is an aldolase having a nucleotide sequence of SEQ ID NO: 22 or an amino acid sequence of SEQ ID NO: 23

DERA 12 is an aldolase having a nucleotide sequence of SEQ ID NO: 24 or an amino acid sequence of SEQ ID NO: 25

DERA 13 is an aldolase having a nucleotide sequence of SEQ ID NO: 26 or an amino acid
sequence of SEQ ID NO: 27
DERA 14 is an aldolase having a nucleotide sequence of SEQ ID NO: 28 or an amino acid sequence of SEQ ID NO: 29
DERA 15 is an aldolase having a nucleotide sequence of SEQ ID NO: 30 or an amino acid sequence of SEQ ID NO: 31
DERA 16 is an aldolase having a nucleotide sequence of SEQ ID NO: 32 or an amino acid sequence of SEQ ID NO: 33
DERA 17 is an aldolase having a nucleotide sequence of SEQ ID NO: 34 or an amino acid sequence of SEQ ID NO: 35
DERA 18 is an aldolase having a nucleotide sequence of SEQ ID NO: 36 or an amino acid sequence of SEQ ID NO: 37
DERA 19 is an aldolase having a nucleotide sequence of SEQ ID NO: 38 or an amino acid sequence of SEQ ID NO: 39
DERA 20 is an aldolase having a nucleotide sequence of SEQ ID NO: 40 or an amino acid sequence of SEQ ID NO: 41
DERA 21 is an aldolase having a nucleotide sequence of SEQ ID NO: 42 or an amino acid sequence of SEQ ID NO: 43
DERA 22 is an aldolase having a nucleotide sequence of SEQ ID NO: 44 or an amino acid sequence of SEQ ID NO: 45
DERA 23 is an aldolase having a nucleotide sequence of SEQ ID NO: 46 or an amino acid sequence of SEQ ID NO: 47

The aldolase encompasses an aldolase having an amino acid sequence identity of at least about 50 % thereof; preferably at least 70 % thereof, to a aldolase described herein. The amino acid sequence identities are determined by the analysis with a sequence comparison algorithm or by a visual inspection. In one aspect the sequence comparison algorithm is made with AlignX algorithm of Vector NTI 9.0 (InforMax) with settings set to default.

In particular, the invention provides for a process comprising the step of reacting a substrate of formula X with acetaldehyde under aldolase-catalysed aldol condensation conditions to form the corresponding lactol I.'
wherein the substrate of formula X

wherein R₂ and R₃ are independently selected from d-C₄-alkyl, or together form a cyclic structure of formula (CH₂)ₙ wherein n is from 2 to 6, and subsequent use thereof in the synthesis of statins (in particular rosvastatin). In this process, in a first embodiment, the aldolase is selected from DERA 01, DERA 06 or DERA 07, or any aldolase having an amino acid sequence identity of at least about 90% to those. In another embodiment, the aldolase is selected from DERA 02 or DERA 15 or DERA 16, or any aldolase having an amino acid sequence identity of at least about 90% to those.

The DERA aldolases described herein can be prepared by any means known in the art, including but not limited to standard protocols for protein expression in recombinant E. coli such as described in Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., Cold Spring Harbor, NY 2001. Modified versions of known DERA aldolases may be necessary or may result depending on cloning conditions and are encompassed by the present invention.

The DERA aldolases described herein can be used in any biologically active form. In one embodiment, the aldolase is active and can be used in the form of a living whole cell catalyst. In another embodiment, the aldolase is active and can be used in the form of an inactivated whole cell catalyst. In yet another embodiment, the aldolase is active and can be used in the form of a homogenized whole cell catalyst. In one embodiment, the aldolase is active and can be used in the form of a cell free extract. In a further embodiment, the aldolase is active and can be used in the form of a purified enzyme by means of any methods known in the art. In another aspect, the aldolase is active and can be used in the form of an extracellularly expressed protein.

Alternatively, the present invention provides a method of preparing intermediates for the production of statins by using a whole cell catalyst. In one embodiment, the whole cell catalyst is any prokaryotic organism over expressing biologically active form of an aldolase. In a particular embodiment, the whole cell catalyst is Escherichia coli over expressing biologically active form of an aldolase.

Substrates and reaction conditions were chosen to give the optimal activity of an aldolase used to make the intermediates useful for a statin production.
The substrates X are in the first aspect selected according to their inhibitory effects towards the aldolase activity. In particular the acceptor substrates with the least inhibitory effect are suitable for the reaction.

The substrates X are also selected according to their product stability and to the corresponding lactol I' product stability at optimal reaction conditions. In particular the acceptor substrates with the best stability are preferred for the reaction.

The substrates X may be in particular 2,2-dimethoxyethanal (preferred), 2,2-diethoxyethanal; 2,2-dipropoxyethanal; 2,2-dibutoxyethanal; 1,3-dioxolane-2-carbaldehyde; 4-methyl-1,3-dioxolane-2-carbaldehyde; 4-ethyl-1,3-dioxolane-2-carbaldehyde; 4-propyl-1,3-dioxolane-2-carbaldehyde; 4-butyl-1,3-dioxolane-2-carbaldehyde. Alternatively the substrates may be the structures which according to conditions of the enzymatic reaction lead to compounds generated in situ, like

![Chemical Structures](attachment:image.png)

wherein substituents B are the same or different selected from OH, d-C₄-alkoxy, halogen and azido groups.

Generally aldolase will be provided in a suitable vessel or reactor, and the substrate of formula X and acetaldehyde will be added batch-wise or continuously.

Specifically aldolase is prepared in an aqueous solution (particularly in a concentration 0.1 g/L to 3 g/L), optionally in the presence of a salt (in particular NaCl in a concentration from 50 to 500 mM). The aqueous solution may contain organic solvents miscible with water (in particular dimethyl sulfoxide in a concentration from 2 to 15 % V/V), and may be buffered to pH 6 to 11. Suitable buffers can be prepared from: acids, bases, salts or mixtures thereof, and any other buffer system known in the art except those possessing a primary, secondary or tertiary amino group. In particular, phosphate buffer, in a concentration 10 to 500 mM can be used. The aqueous solution can also be prepared by adding the aldolase to water and maintaining the pH-value during the reaction by means of an automated addition of inorganic acids, bases, salts or
mixtures thereof.

In the process aspect, the substrate of formula X may be added to the reaction mixture continuously or alternatively the substrate of formula X may be added to the reaction mixture in one batch or more batches. In one aspect, the total amount of substrates added to the mixture is such that the total amount of the substrate (I) added would be from about 20 mmol per liter of the reaction mixture to about 2 mol per liter of the reaction mixture, in particular from about 100 mmol per liter of the reaction mixture to about 1.5 mmol per liter of the reaction mixture, more particular from about 200 mmol per liter of the reaction mixture to about 700 mmol per liter of the reaction mixture. Acetaldehyde may be added by several means. In one aspect, acetaldehyde is added to the reaction mixture in one batch or more batches or alternatively continuously. Acetaldehyde may be premixed with the substrate of formula X and added to the reaction mixture. The total amount of acetaldehyde added to the reaction mixture is from about 0.1 to about 4 molar equivalents to the total amount of the acceptor substrate, in particular from about 2 to about 2.5 molar equivalents.

Alternatively aldolase may be added to the reaction mixture containing a solvent and at least one of the substrate of formula X or acetaldehyde. The substrate X or acetaldehyde which is not comprised in the provided reaction mixture may be added together with aldolase or after the addition of aldolase.

In one aspect of the invention, the pH-value used for the aldolase-catalyzed reaction is from about 5 to about 12. In one embodiment, the pH used for aldolase-catalyzed reaction is from about 6 to about 10. In another embodiment, the pH-value used for aldolase-catalyzed reaction is from about 7 to about 9. Specifically the pH-value will be maintained by a suitable buffer in a range from 7.2 to 8.5.

Some commonly used buffers can lower the yield of the aforementioned aldolase-catalysed reaction by limiting the availability of aldolase-condensation intermediates, particularly first condensation reaction products as they may undergo a chemical reaction with a buffer. We discovered that bis-tris propane reacts with said intermediates ((S)-3-hydroxy-4,4-dimethoxybutanal) giving (S,2)-2-(3-((1,3-dihydroxy-2-hydroxymethyl)propan-2-yl)(3-hydroxy-4,4-dimethoxybut-1-enyl)amino)propylamino)-2-(hydroxymethyl)propane-1,3-diol. Other buffers that may react similarly are bis-tris, tricin, tris, bicin or any other buffer having a primary, secondary or tertiary amino group. Thus suitable buffers for adjusting pH, if this adjustment is needed, are made with acids, bases, salts or mixtures thereof, in particular
phosphoric acid and sodium hydroxide. In a particularly preferred embodiment, the buffer is a phosphate buffer.

In one aspect according to the invention, the temperature used for the aldolase-catalyzed reaction is from about 20 to about 70 °C. In one embodiment, the temperature used for aldolase-catalyzed reaction is from about 25 to about 60 °C. In another embodiment, the temperature used for aldolase-catalyzed reaction is from about 30 to about 50 °C.

The reaction is industrially suitable, as it proceeds to completion within few hours.

The effects of reaction conditions, notably pH, temperature and reaction time are surprising, especially in view of the teaching of J. Am. Chem. Soc., 117, 29, (1995) p. 7585, where the reaction with 2,2-dimethoxyethanal did not proceed even in 6 days.

After the completion of the reaction, the enzyme is removed from the reaction mixture, for example by the addition of at least about 1 vol. of acetonitrile to 1 vol. of reaction mixture. Alternatively the enzyme is removed by any salting out method known in the art. In one embodiment the salting out is performed with the addition of ammonium sulfate of at least 5 % m/V.

The invention also provides a purification method for obtaining pure lactols produced by the aldolase-catalysed aldol condensation reaction. In one aspect, acetonitrile is evaporated from the reaction mixture and the remaining aqueous solution is then lyophilised. In another aspect, the salting-out solution is lyophilised. The powdered remainder is then suspended in MTBE (methyl-t-butylether) / methanol 1:1. The suspension is filtered to remove insoluble salts and the filtrate is loaded to a silicagel column by using MTBE/Methanol 1:1 as the mobile phase.

In a particular embodiment, the invention provides for the reaction of 2,2-dimethoxyethanal with acetaldehyde under aldolase-catalysed aldol condensation to form (4R,6S)-6-dimethoxymethyl-tetrahydro-2H-pyran-2,4-diol wherein the used aldolase is DERA 01, DERA 02 or DERA 08 in an appropriate solvent (in particular an aqueous solvent, which may be water in a mixture with a water soluble organic solvent) in a pH range from 6 to 11, in particular from 7 to 9 (adjusted if needed with acids, bases, salts or mixtures thereof in particular with phosphoric acid and sodium hydroxide). The reaction proceeds at a temperature around 35-40 °C and the conversion is finished in 1 to 6 hours.

In general, the aldolase used is prepared by methods of protein expression described in

The reaction yields are calculated relatively to the total amount of the substrate of formula X added to the reaction mixtures and they are determined as the ratio between moles of the isolated product and moles of the substrate of formula X added to the reaction mixture.

The following examples illustrate the process of the present invention and are not intended to limit the scope of the invention.

Example 1 (Synthesis of the standard)

1. Step: Preparation of

\[
((2S,4R)-4-(\text{tert-butyldimethylsilyloxy})-6-\text{oxo-tetrahydro-2H-pyran-2-yl})\text{methyl acetate}
\]

To a solution of \((4f,6S)-4-(\text{te/t-butyldimethylsilyloxy})-6-\text{(iodomethyl)}\text{-tetrahydropyran-2-one}
\) (40.00 g, 108.0 mmol) in AcOH (660 mL), AgOAc (20.03 g, 118.8 mmol) is added. The resultant mixture is then heated at 125 °C for 6 hours. The reaction mixture is filtered through diatomite filter medium (Celite®). The obtained filtrate is evaporated to afford the residue. To this residue, EtOAc (500 mL) and water (600 mL) are added. The organic layer is separated and the aqueous layer is washed again with EtOAc (5 x 150 mL). The combined organic layers are washed with water (4 x 300 mL), brine (5 x 300 mL) and dried over anhydrous MgSO₄, filtered and concentrated under the reduced pressure to afford 30.28 g (92.6 %) of \(((2S,4R)-4-(\text{tert-butyldimethylsilyloxy})-6-\text{oxo-tetrahydro-2H-pyran-2-yl})\text{methyl acetate}
\) as yellow oil (HPLC purity 98 %).

\[\text{H-NMR} \ (300 \text{ MHz}, \text{CDCl}_3) \ \delta: \ 4.93 \ (m, \ 1H), \ 4.37 \ (m, \ 1H), \ 4.30 \ (dd, \ J = 12 \ Hz, \ J = 3 \ Hz, \ 1H), 4.21 \ (dd, \ J = 12 \ Hz, \ J = 5 \ Hz, \ 1H), \ 2.62 \ (d, \ J = 4 \ Hz , \ 2H), \ 2.11 \ (s, \ 3H), \ 1.84-1.80 \ (m, \ 2H), \ 0.89 \ (s, \ 9H), \ 0.09, \ 0.09 \ (2s, \ 6H).\]

\[\text{C-NMR} \ (75 \text{ MHz}, \text{CDCl}_3) \ \delta: \ 170.4, \ 169.1, \ 173.3, \ 65.5, \ 63.0, \ 38.9, \ 32.2, \ 20.5, \ 17.7, \ -5.1, \ -5.2.\]

2. Step: Preparation of

\[(4f,6S)-4-(\text{tert-butyldimethylsilyloxy})-6-(\text{hydroxymethyl})\text{-tetrahydropyran-2-one}\]

\[\text{([Bu}_{2}\text{SnOH(Cl)]}_2 \ (1.577 g, \ 2.764 \text{ mmol}) \ are dissolved in MeOH/THF mixture (280 mL). The reaction mixture is stirred at 23-25 °C for 27 h. After the solvent is removed under reduced pressure, the remained residue is purified by silica gel chromatography (elution}
\]
with 'BuMeO/hexane mixture) to afford a crude product as white solid (5.59 g, 78%).
Recrystallization from n-hexane affords (3.90 g, 54 %) of (4/?',6S)-4-(tert-butyldimethylsilyloxy)-
6-(hydroxymethyl)-tetrahydro-pyran-2-one as white needles. M.p. = 102 °C (DSC peak).

$^1$H-NMR (300 MHz, CDCl$_3$) $\delta$: 4.80 (m, 1H), 4.38 (m, 1H), 3.91 (dd, J = 12 Hz, J = 3 Hz, 1 H),
3.66 (dd, J = 12 Hz, J = 5 Hz, 1 H), 2.60 (d, J = 4 Hz , 2H), 2.31 (bs, 1H), 1.97-1.75 (m, 2H), 0.88
(s, 9H), 0.09, 0.08 (2s, 6H).

$^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$: 170.1, 76.8, 64.7, 63.4, 39.2, 31.9, 25.6, 17.9, -4.9, -5.0.

3. Step: Preparation of

(4ff,6S)-4-(tert-butyldimethylsilyloxy)-6-(dihydroxymethyl)tetrahydro-2H-pyran-2-one (V)

A mixture of (4ff,6S)-4-(tert-butyldimethylsilyloxy)-6-(hydroxymethyl)-tetrahydropyran-2-one
(150 mg, 0.58 mmol) and Dess-Martin periodinane (380 mg, 0.86 mmol) in CH$_2$Cl$_2$ (15 mL) is
stirred at ambient temperature for 3 hours. The mixture is diluted with 'BuMeO (20 mL), washed
with saturated Na$_2$S$_2$O$_3$ solution, saturated NaHCO$_3$ solution, dried (MgSO$_4$) and concentrated
to give 139 mg (87 %) of (4/?,6S)-4-(tert-butyldimethylsilyloxy)-6-(dihydroxy methyl)tetrahydro-
2H-pyran-2-one as white powder which is used in the next step without further purification.

$^1$H-NMR (300 MHz, THF-d$_8$) $\delta$: 5.27 (d, J = 6 Hz, 1H, OH), 5.1 9 (d, J = 6 Hz, 1H, OH), 4.90-4.85
(m, 1H), 4.44-4.38 (m, 2H), 2.58 (dd, J = 17 Hz, J = 4 Hz, 1H), 2.44-2.36 (m, 1H), 1.92-1.87 (m, 2H), 0.91 (s, 9H), 0.1 0 (s, 6H).

$^{13}$C-NMR (75 MHz, THF-d$_8$) $\delta$: 168.7, 91.7, 79.0, 65.1 , 40.3, 31.0, 26.2, 18.7, -4.8, -4.8.

4. Step: Preparation of

(4ff,6S)-4-(tert-butyldimethylsilyloxy)-6-(dimethoxymethyl)tetrahydro-2H-pyran-2-one (Iir)

To a solution of 1.0 g of (4ff,6S)-4-(te/t-butyldimethylsilyloxy)-6-(dihydroxymethyl)-
tetrahydropyran-2-one (V) in 100 mL of dichloromethane, 50 mg of toluenesulfonic acid and 4.5
mL of trimethyl orthoformate are added. After 2 hours of stirring at 25 °C, 0.2 g of NaHCO$_3$ is
added and dichloromethane is distilled off. The residue is flash chromatographed
(MTBE/hexane 1/1) and the solvents are distilled off to get the title compound.

Yield: 0.70 g of yellow, crystalline powder.

$^1$H-NMR (300 MHz, CDCl$_3$) $\delta$: 0.08 (s), 0.88 (s), 1.8 - 1.9 (m), 2.5-2.7 (m), 3.44 (s), 3.45 (s),
4.36 (t), 4.42 (d), 4.73 (m).

$^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$: -5.0, 17.9, 25.6, 26.9, 29.7, 39.5, 55.8, 56.8, 63.3, 75.7, 105.1 ,
169.5.

5. Step: Preparation of
(4R,6S)-4-(tert-butyldimethylsilyloxy)-6-(dimethoxymethyl)-tetrahydro-2H-pyran-2-ol \( (I') \)

A solution of 0.6 g of (4R,6S)-4-(tert-butyldimethylsilyloxy)-6-(dimethoxymethyl)-tetrahydropyran-2-one \( (I') \) in 36 mL of dichloromethane is cooled to -78 °C after 2 mL of DIBALH (25 % in toluene) are added over 10 minutes. After 1 hour of stirring at -78 °C, 0.9 g of Rochelle salt in 60 mL of water are added, phases are separated and dichloromethane is distilled off. The residue is flash chromatographed (MTBE/hexane 1/1) and the solvents are distilled off to get the title compound.

Yield: 0.47 g of yellow oil.

Mixture of α and β anomers:

\(^{1}H\)-NMR (300 MHz, CDCl₃) \( \delta \): 0.10 (s), 0.86 (s), 0.89 (s), 1.4-1.9 (m), 3.35 (s), 3.42 (s), 4.02 (m), 4.2-4.4 (m), 5.13 (d), 5.23 (d), 5.51 (d).

\(^{13}C\)-NMR (75 MHz, CDCl₃) \( \delta \): -5.6, -5.2, 17.7, 17.8, 25.5, 32.1, 33.2, 36.0, 40.1, 53.8, 54.2, 54.6, 55.0, 92.7, 92.8, 105.5, 105.6.

6. Step: Preparation of the standard,

(4R,6S)-6-(dimethoxymethyl)-tetrahydro-2H-pyran-2,4-diol \( (I'') \)

To a solution of 0.47 g of (4R,6S)-4-(tert-butyldimethylsilyloxy)-6-(dimethoxymethyl)-tetrahydro-2H-pyran-2-ol \( (I') \) in 14 mL of tetrahydrofurane, tetra-n-butylammonium fluoride in 14 mL of THF is added. After 15 minutes of stirring at 25 °C, the THF is distilled off. The residue is flash chromatographed (methanol). After methanol is distilled off, the title compound is obtained as an oil.

Yield: 0.18 g of yellow oil.

Mixture of α and β anomers:

\(^{1}H\)-NMR : (300 MHz, CDCl₃) \( \delta \): 1.4 - 2.1 (m), 3.44 (s), 3.45 (s), 4.05 (m), 4.20 - 4.50 (m), 5.20 (dd), 5.38 (d).

\(^{13}C\)-NMR : (300 MHz, CDCl₃) \( \delta \): 32.8, 35.0, 54.4, 54.5, 54.9, 55.0, 62.8, 64.5, 64.9, 70.4, 92.5, 92.9, 105.4, 105.7.

Example 2

Example 2.1 : Preparation of aldolase

Escherichia coli gene deoC has been amplified by using oligonucleotide primers

\[ CGGGATCCACTGATCTGAAAGCAAGCAGCC \] (SEQ ID NO: 48) and

\[ GCAAGCTTGCTGCTGGCGCTCTTACC \] (SEQ ID NO: 49) in a PCR reaction using isolated genome DNA from \textit{E. coli} K-12 strain. The product was cleaved with restriction endonucleases
BamHI and HindIII and the resulting fragment has been separated on agarose gel electrophoresis and purified. An expression vector pQE30 (Qiagene inc., Valencia, CA, USA) has been cleaved by using the same aforementioned restriction endonucleases and purified. The fragments have been assembled in a T4 ligase reaction. Competent Escherichia coli DH5α cells were transformed with the above mentioned ligation reaction. Ampicilin resistance colonies were cultured and plasmid DNA has been isolated. The resulting construct has been designated pQE30DeraC and sequenced for conformation of the gene sequence. Aldolase expressing organism has been prepared by transforming competent Escherichia coli TOP10 F' strain (Invitrogen corp., Carlsbad, CA, USA) with vector pQE30DeraC. The methods used for the process are described in Sambrook, et al. (1989) Molecular cloning: A laboratory Manual 2nd Edition, New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor and are well known to a person skilled in the art.

Terrific Broth media (150 mL, 12 g/L bacto tryptone, 24 g/L bacto yeast extract, 4 mL/L glycerol, 2.31 g/L KH₂PO₄, 12.54 g/L K₂HPO₄) supplemented with ampicillin (100 μg/mL) was inoculated with 3 mL of TOP10 F' pQE30DeraC overnight culture. Cells were grown at 37 °C, 250 rpm until OD₆₀₀ reached approx. 0.8. Protein expression was induced with IPTG (1 mM final concentration) and cells were left in the same growing conditions for additional 4 h. The cell pellet was harvested by centrifugation (10 min, 6000 g, 4 °C) and the pellet was resuspended in phosphate buffer (9 mL, 50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl) giving whole cell catalyst. Alternatively the pellet was resuspended in lytic buffer (9 mL, 50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl, 2 mM DTT). Cells were sonified (3 x 15 s) by using Branson digital sonifier and cell debris was removed by sedimentation (10 min, 20 000 g, 4 °C). Clear aqueous solution of DERA 01 was thus obtained.

Example 2.2: Aldolase catalyzed aldol condensation conditions

Example 2.2.1:
Preparation of (4ff,6S)-6-(dimethoxymethyl)tetrahydro-2H-pyran-2,4-diol

\[
\begin{align*}
\text{X'} & \quad + \quad 2 \quad \text{CH₃CHO} \quad \xrightarrow{\text{DERA 01}} \quad \text{I''} \\
\text{DERA 01} & \quad \xrightarrow{\text{3 h, 37 °C}} \\
\end{align*}
\]

To a solution of phosphate buffer (700 mL, 50 mM NaH₂PO₄, pH 8.0, 300mM NaCl) and 2,2-dimethoxyethanal X' (100 mL, 0.5 M in phosphate buffer, as described above), an aqueous solution of DERA 01 (100 ml, prepared according to Example 2.1) and a solution of acetaldehyde (Fluka, 100 mL, 1.0 M in phosphate buffer as described above) was added. The mixture was stirred for 3 h at 37 °C and pH was regulated at 8.0 with NaOH. The conversion of
the reaction was monitored by gas chromatography (GC). After 3 h, the proteins in the reaction mixture (1 L) were precipitated with acetonitrile (4 L) and the solution was filtered by using celite and glass filter. The acetonitrile was evaporated and residual water was removed with lyophilization to give the crude lactol I′ (18 g), which was directly solubilized, filtered and submitted to a silica-gel column (methanol / /-butyl methyl ether 1:1). Fractions were collected and analyzed by thin-layer silica-gel chromatography (diisopropyl ether / acetonitrile 2:1). The solvent from the fraction with the product I′ was evaporated and the dried product (154 mg, 1.6 % yield) was analyzed by 1H- and 13C-NMR. Data for the major anomer:

1H-NMR (300 MHz, CDCl3): δ = 1.4-1.9 (m), 3.42 (s), 4.15-4.35 (m), 5.25 (d).

13C-NMR (300 MHz, CDCl3): δ = 32.8, 35.0, 54.5, 54.9, 62.8, 64.5, 92.9, 105.4, 105.7.

Example 2.2.2:
Preparation of (4ff,6S)-6-(dimethoxymethyl)tetrahydro-2H-pyran-2,4-diol (I′′)

\[
\begin{align*}
\text{O} & \quad \text{H} \\
\text{O} & \quad \text{H} \\
\text{X}′ & \quad 2 \quad \text{H} & \quad \text{DERA 02} & \quad 3 \text{ h, } 37 \text{ °C} & \quad \text{OH} \\
\text{I}″ & \quad \text{OH}
\end{align*}
\]

To a solution of phosphate buffer (700 mL, 50 mM NaH2PO4, pH 8.0, 300 mM NaCl) and 2,2-dimethoxyethanol (100 mL, 0.5 M in phosphate buffer, as described above), an aqueous solution of DERA 02 (100 ml, prepared by an analogue method to the one described in Example 2.1) and a solution of acetaldehyde (Fluka, 100 mL, 1.0 M in phosphate buffer, as described above) was added. The mixture was stirred for 3 h at 37 °C and the pH was kept at 8.0. The conversion of the reaction was monitored by gas chromatography (GC). After 3 h, the proteins in the reaction mixture (1 L) were precipitated with acetonitrile (4 L) and the solution was filtered by using celite and glass filter. Acetonitrile was evaporated and residual water was removed with lyophilization to give the crude lactol I′ (22 g), which was directly solubilized, filtered and submitted to a silica-gel column (methanol / /-butyl methyl ether 1:1). Fractions were collected and analyzed by thin-layer silica-gel chromatography (diisopropyl ether / acetonitrile 2:1). The solvent from the fraction with the product I′ was evaporated and the dried product (2.8 g, 29 % yield) was analyzed by 1H- and 13C-NMR. Data for the major anomer:

1H-NMR (300 MHz, CDCl3): δ = 1.4-1.9 (m), 3.42 (s), 4.15-4.35 (m), 5.25 (d).

13C-NMR (300 MHz, CDCl3): δ = 32.8, 35.0, 54.5, 54.9, 62.8, 64.5, 92.9, 105.4, 105.7.

Example 2.2.3
Preparation of (4ff,6S)-6-(dimethoxymethyl)tetrahydro-2H-pyran-2,4-diol (I″)
To a solution of phosphate buffer (700 ml, 50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl) and 2,2-dimethoxyethanal (100 ml, 1.0M in phosphate buffer, as described above), a whole cell catalyst with DERA 02 (100 ml, prepared by an analogue method to the one described in Example 2.1) and a solution of acetaldehyde (Fluka, 100 ml, 2.0 M in phosphate buffer, as described above) was added. The mixture was stirred for 3 h at 37 °C and pH was kept at 8.0. The conversion of the reaction was monitored by gas chromatography (GC). After 3 h, the cells were removed by sedimentation (10 min, 6000 g, 4 °C). Residual water was removed by lyophilization to give the crude lactol I" (23 g) as an oil, which was directly solubilized, filtered and submitted to a silica-gel column (methanol / /-butyl methyl ether 1:1). Fractions were collected and analyzed by thin-layer silica-gel chromatography (disisopropyl ether / acetonitrile 2:1). The solvent from the fraction with the product I" was evaporated and the dried product (1.8 g, 9.4 % yield) was analyzed by ¹H- and ¹³C-NMR.

¹H-NMR (300 MHz, CDCl₃): δ = 1.4-1.9 (m), 3.42 (s), 4.15-4.35 (m), 5.25 (d).

¹³C-NMR (300 MHz, CDCl₃): δ = 32.8, 35.0, 54.5, 54.9, 62.8, 64.5, 92.9, 105.4, 105.7.

Example 2.2.4:
Preparation of (4ff,6S)-6-(dimethoxymethyl)tetrahydro-2H-pyran-2,4-diol (I")

To a solution of phosphate buffer (700 ml, 50 mM NaH₂PO₄, pH 8.0), an aqueous solution of DERA 02 (100 ml, prepared by an analogue method to the one described in Example 2.1) was added. A solution of 2,2-dimethoxyethanal (Aldrich, 100 ml, 1.0 M in phosphate buffer, as described above) and acetaldehyde (Fluka, 100 ml, 2.0 M in phosphate buffer, as described above) was continuously added separately to the above DERA solution by a programmed pump within 2 h. The mixture was stirred for 3 h at 37 °C and pH was kept at 8.0. The conversion of the reaction was monitored by gas chromatography (GC). After 3 h, the proteins in the reaction mixture (1 L) were precipitated with acetonitrile (4 L) and the solution was filtered by using celite and glass filter. Acetonitrile was evaporated and residual water was removed by lyophilization to give the crude lactol I" (15 g), which was directly solubilized, filtered and submitted to a silica-gel column (methanol / /-butyl methyl ether 1:1). Fractions were collected and analyzed by thin-
layer silica-gel chromatography (diisopropyl ether / acetonitrile 2:1). The solvent from the fraction with the product I'' was evaporated and the dried product (6.2 g, 23.3 % yield) was analyzed by $^1$H- and $^{13}$C-NMR.

$^1$H-NMR (300 MHz, CDCl$_3$): δ = 1.4-1.9 (m), 3.42 (s), 4.15-4.35 (m), 5.25 (d).

$^{13}$C-NMR (300 MHz, CDCl$_3$): δ = 32.8, 35.0, 54.5, 54.9, 62.8, 64.5, 92.9, 105.4, 105.7.

**Example 2.2.5:**

**Preparation of (4f,6S)-6-(dimethoxymethyl)tetrahydro-2W-pyran-2,4-diol (I'')**

\[
\begin{align*}
\text{X'} & \quad \text{DERA} \quad \text{H} \quad \text{C} \quad \text{O} \\
\text{OH} & \quad 3 \text{h} \quad 37^\circ \text{C} \\
\text{OH} & \quad \text{I''}
\end{align*}
\]

To a solution of phosphate buffer (700 ml, 50 mM NaH$_2$PO$_4$, pH 8.0), an aqueous solution of DERA 02 (100 ml, prepared by an analogue method to the one described in Example 1) and a solution of 2,2-dimethoxyethanal (Aldrich, 100 ml, 4.0 M in phosphate buffer, as described above) was added. A solution of acetaldehyde (Fluka, 100 ml, 8.0 M in phosphate buffer, as described above) was continuously added to the above DERA solution by a programmed pump within 2 h. The mixture was stirred for 3 h at 37 °C and pH was kept at 8.0. The conversion of the reaction was monitored by gas chromatography (GC). After 3 h the proteins in the reaction mixture (1 L) were precipitated with acetonitrile (4 L) and the solution was filtered by using celite and glass filter. Acetonitrile was evaporated and residual water was removed by lyophilization to give the crude lactol I'' (37 g), which was directly solubilized, filtered and submitted to a silica-gel column (methanol / *-butyl methyl ether 1:1). Fractions were collected and analyzed by thin-layer silica-gel chromatography (diisopropyl ether / acetonitrile 2:1). The solvent from the fraction with the product I'' was evaporated and the dried product (9.4 g, 12.2 % yield) was analyzed by $^1$H- and $^{13}$C-NMR.

$^1$H-NMR (300 MHz, CDCl$_3$): δ = 1.4-1.9 (m), 3.42 (s), 4.15-4.35 (m), 5.25 (d).

$^{13}$C-NMR (300 MHz, CDCl$_3$): δ = 32.8, 35.0, 54.5, 54.9, 62.8, 64.5, 92.9, 105.4, 105.7.

**Example 2.3: Preparation of Rosuvastatin**

To a solution of 1.4 g of (4R,6S)-6-(dimethoxymethyl)-tetrahydro-2H-pyran-2,4-diol (I'') in 100 mL of water is added 2.4 g barium carbonate and 0.5 mL of bromine at 0 °C. After 3 hours of stirring at 0 - 5 °C, the product is extracted three times with 300 mL of ethyl acetate. Finally the ethyl acetate is distilled off. To the 0.5 g of thus prepared (4R,6S)-6-(dimethoxymethyl)-4-hydroxy-tetrahydropyran-2-one (M') in 50 mL of dichloromethane, a 280 mg of imidazole are added at 25 °C and after cooling to 0 °C, 400 mg of tert-butylidimethylsilyl chloride are added.
The reaction is then stirred for 18 hours and dichloromethane is distilled off. To a solution of 0.40 g of thus obtained (4R,6S)-4-(tert-butyldimethylsilyloxy)-6-(dimethoxymethyl)-tetrahydropyran-2-one (IH') 35 mg of iodine in 12 mL of acetone are added at 25 °C and stirred for 48 hours. In the next step, 200 mg of the obtained (4R,6S)-4-(tert-butyldimethylsilyloxy)-6-(dihydroxymethyl)tetrahydro-2H-pyran-2-one (IV) are dissolved in toluene and added to a stirred suspension of ((4-(4-fluorophenyl)-6-isopropyl-2-(N-methylmethyl-sulfonamido)pyrimidin-5-yl)methyl)tributylphosphonium 2,2,2-trifluoro-acetate (VII) (504 mg) and sodium hexamethyl disilazane in dry toluene. The protecting group is removed by tetra-n-butylammonium fluoride and the obtained compound VIII is converted to the corresponding calcium salt IX by calcium acetate.

Example 2.4: Preparation of
(S,Z)-2-(3-((1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl)(3-hydroxy-4,4-dimethoxybut-1-enyl)amino)propylamino)-2-(hydroxymethyl)propane-1,3-diol [1]

\[
\begin{align*}
\text{X'} & \quad \text{[2]} \\
\text{[1]} & \quad \text{[1]}
\end{align*}
\]

To a solution of bis-tris propane [2] buffer (70 mL, 50 mM bis-tris propane, pH 8.0, 300 mM NaCl) and a solution of 2,2-dimethoxyethanal X' (10 mL, 0.5 M in bis-tris propane buffer as described above), an aqueous solution of DERA 02 (10 mL, prepared with analogue method to one described in Example 1) and a solution of acetaldehyde (10 mL, 1.0 M in bis-tris propane buffer as described above) was added. The mixture was stirred for 3 h at 37 °C and pH was regulated at 8.0 with NaOH. After 3 h the proteins in the sample (1 mL) from the reaction mixture (100 mL) were precipitated with acetonitrile (9 mL) and the solution was centrifuged. A sample from the supernatant was collected and analyzed directly by HPLC-MS/MS instrument: HPLC-MS/MS m/z[M+H]^+ 413.

Example 3

Step a) : Preparation of
(4ff,6S)-6-(dimethoxymethyl)-4-hydroxy-tetrahydropyran-2-one (IH')
To a solution of 1.4 g of (4'/r,S)-6-(dimethoxymethyl)-tetrahydro-2/-/-pyran-2,4-diol (I') in 100 mL of water, 2.4 g of barium carbonate and 0.5 mL of bromine at O °C are added. After 3 hours of stirring at O-5 °C, the product II' is extracted three times with 300 mL of ethyl acetate. Finally ethyl acetate is distilled off. The residue is flash chromatographed (DIPE/acetonitrile 2/1). The solvents are distilled off to get the title compound.

Yield: 0.7 g of yellow oil.

\[ ^1H-NMR \ (300 \text{ MHz, CDCl}_3) \delta: 1.8-1.9 \text{ (m), 2.5-2.7 \text{ (m), 3.40 \text{ (s), 3.41 \text{ (s), 4.3-4.4 \text{ (m), 4.6-4.7 \text{ (m).}}}} \]

\[ ^{13}C-NMR \ (75 \text{ MHz, CDCl}_3) \delta: 29.3, 38.7, 55.8, 56.9, 62.4, 75.5, 105.0, 169.8. \]

**Step b): Preparation of (4ff,6S)-4-(tert-butyldimethylsilyloxy)-6-(dimethoxymethyl)-tetrahydropyran-2-one (III')**

To a solution of 0.5 g of (4'/r,S)-6-(dimethoxymethyl)-4-hydroxy-tetrahydropyran-2-one (M') in 50 mL of dichloromethane, 280 mg of imidazole are added at 25 °C. After cooling to O °C, 400 mg of te/t-butyldimethylsilyl chloride are added. The reaction mixture is then stirred for 18 hours and dichloromethane is distilled off. The residue is flash chromatographed (DIPE/acetonitrile = 5/1). The solvents are distilled off to get the title compound.

Yield: 0.40 g of yellow, crystalline powder.

\[ ^1H-NMR \ (300 \text{ MHz, CDCl}_3) \delta: 0.08 \text{ (s), 0.88 \text{ (s), 1.9 \text{ (m), 2.6 \text{ (m), 3.44 \text{ (s), 3.45 \text{ (s), 4.36 \text{ (t), 4.42 \text{ (d), 4.73 \text{ (m).}}}}}} \]

\[ ^{13}C-NMR \ (75 \text{ MHz, CDCl}_3) \delta: -5.0, 17.9, 25.6, 26.9, 29.7, 39.5, 55.8, 56.8, 63.3, 75.7, 105.1, 169.5. \]
Step c): Preparation of (4fl,6S)-4-(tert-butyldimethylsilyloxy)-6-(dihydroxymethyl)-tetrahydropyran-2-one (V’) and (2S,4fl)-4-(tert-butyldimethylsilyloxy)-6-oxo-tetrahydro-2H-pyran-2-carbaldehyde (IV)

To a solution of 0.40 g of (4fl,6S)-4-(te/t-butyldimethylsilyloxy)-6-(dimethoxymethyl)-tetrahydropyran-2-one (I'H) in 12 ml of acetone, 35 mg of iodine are added at 25 °C. The reaction mixture is then stirred for 48 hours and acetone is distilled off. Thereafter 25 ml of dichloromethane is added and the reaction mixture is washed with 20 ml of a saturated sodium thiosulfate solution and 20 ml of water. The organic layer is separated and the organic phase is distilled off to get the compound (V).

Yield: 0.25 g of white powder.

The equilibrium between (2S,4fl)-4-(te/t-butyldimethylsilyloxy)-6-oxo-tetrahydro-2/-/-pyran-2-carbaldehyde (IV) and its hydrate (V) as studied by NMR spectroscopy proves that in chlorinated hydrocarbons: chloroform, dichloromethane; hydrocarbons: pentane, hexane, heptane, methylcyclohexane and cyclohexane and particularly in aromatic hydrocarbons: toluene (or its chlorinated analogues) the equilibrium is shifted completely towards aldehyde.

Use of toluene as the solvent for the Wittig reaction significantly increases the yield compared to the commonly used THF because in THF aldehyde and hydrate are present in approximately equal amounts while aromatic hydrocarbons favour the aldehyde.

The isolated hydrate form of aldehyde has the following NMR spectra:

$^1$H-NMR (300 MHz, THF-d$_8$) δ: 5.27 (d, J = 6 Hz, 1H, OH), 5.19 (d, J = 6 Hz, 1H, OH), 4.90-4.85 (m, 1H), 4.44-4.38 (m, 2H), 2.58 (dd, J = 17 Hz, J = 4 Hz, 1H), 2.44-2.36 (m, 1H), 1.92-1.87 (m, 2H), 0.91 (s, 9H), 0.10 (s, 6H).

$^{13}$C-NMR (75 MHz, THF-d$_8$) δ: 168.7, 91.7, 79.0, 65.1, 40.3, 31.0, 26.2, 18.7, -4.8, -4.8.

The aldehyde formed in an aprotic solvent has the following NMR spectra:

$^1$H-NMR (300 MHz, CDCl$_3$) δ: 9.82 (s, 1H), 5.09 (dd, J = 11 Hz, J = 4 Hz, 1H), 4.38 (m, 1H), 2.67 (d, J = 4 Hz, 2H), 2.18-2.10 (m, 1H), 1.91-1.81 (m, 1H), 0.89 (s, 9H), 0.09 (s, 6H).
The obtained \(^{(4f,6S)}\)-4-(te/t-butylidemethylsilyloxy)-6-(dihydroxymethyl)tetrahydro-2H-pyran-2-one \((V)\) is used in the next step without further purification by dissolution in toluene where dehydration to \((2S,4^/-?)\)-4-\((te/t-butylidemethylsilyloxy)-6-oxo-tetrahydro-2/-/-pyran-2-carbaldehyde \((IV)\) occurs.

**Example 4**

**Example 4.1 : Preparation of**

\(\Lambda^'-((5-((\Lambda^-)2-\((2S,4^/-4-(tert-butylidemethylsilyloxy)-6-oxotetrahydro-2W-pyran-2-yl)vinyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-\Lambda^-\)-methylmethanesulphonamide \((\text{VI}')\)

To a stirred suspension of \(\((4-(4-fluorophenyl)-6-isopropyl-2-(\Lambda^-\)-methylmethylsulphonamido)pyrimidin-5-yl)methyl\)tributylphosphonium \((4\text{-fluorophenyl})-6\text{-isopropylpyrimidin-2-yl}-(\text{V})\) \((200\text{ mg, 0.77 mmol})\) in 25 ml of dry toluene obtained by dissolution of \((4f,6S)-4-(\text{te/t-butylidemethylsilyloxy})-6-(\text{dihydroxymethyl})\)-tetrahydropran-2-one \((\text{V})\) \((214\text{ mg, 0.77 mmol})\) in toluene and removal of released water. After 24 hours of stirring at room temperature the solution is treated with saturated ammonium chloride solution or water. The aqueous phase is extracted with 'BuMeO (2 x 20 ml, and the combined organic layers dried and concentrated. The residue is purified by silica gel chromatography (elution with 'BuMeO/hexane mixture) to give 273 mg \((61\%)\) of \(\Lambda^-\)-(5-\((\Lambda^-)\)-2-\((2S,4^/-?)\)-4-\((\text{te/t-butylidemethylsilyloxy})-6-\text{oxo-tetrahydro-2/-/-pyran-2-yl})\)-vinyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-\Lambda^-\)-methylmethanesulphonamide as a white solid.

**Example 4.2: Preparation of the calcium salt of \((3ff,5S,\Lambda^-)\)-7-(4-(4-fluorophenyl)-6-isopropyl-2-(\Lambda^-\)-methylmethylsulphonamidopyrimidin-5-yl)-3,5-dihydroxyhept-6-enoic acid \((\text{IX})\)**
To a stirred solution of \(\mathcal{L}\)-(5-((£)-2-((2S,4R)-4-(Butyl)methanlsilyloxy)-6-oxo-tetrahydro-2H-pyran-2-yl)(vinyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl))-\(\mathcal{L}\)-methylmethanesulfonamide (VM') (190 mg, 0.33 mmol) in 3 ml_ of tetrahydrofurane, a solution of acetic acid (55 mg, 0.92 mmol) and tetra-n-butylammonium fluoride trihydrate (183 mg, 0.58 mmol) in 3 ml_ of tetrahydrofurane is added. The reaction mixture is stirred at 20 - 30 °C for 48 h, treated with 10 ml_ of water and extracted several times with 'BuMeO. The combined organic layers are washed successively with the saturated NaHCO₃ solution, water and brine, dried with Na₂SO₄ and concentrated under the reduced pressure. The residue is dissolved in 3 ml_ of a 4:1 mixture of THF/H₂O. The clear solution is warmed to 30 °C and 8.0 M NaOH (0.044 ml_, 0.35 mmol) is added portionwise. The reaction mixture is stirred at 30 °C for 2 hours giving a clear yellow solution. Then THF is removed completely under the reduced pressure (20 mbar) at 40 °C. The remaining water solution is diluted with H₂O to 1.5 ml_ and washed with AcOEt (2x1 ml_). After separation from the organic layer, the aqueous phase is distilled under the reduced pressure (20 mbar) at 40 °C to completely remove the dissolved AcOEt. The remaining clear solution of sodium rosuvastatinate (1.3 ml_) is diluted with H₂O to 1.5 ml_ and warmed to 40 °C. To a vigorously stirred solution of sodium rosuvastatinate is added dropwise Ca(OAc)₂·3H₂O (44 mg, 0.25 mmol in 0.3 ml_ of H₂O) over 5 minutes at 40 °C to precipitate rosuvastatin calcium (IX). After the complete addition, the suspension is stirred further for 30 minutes at 40 °C. The white precipitate is filtered off. Then the wet white solid is suspended in H₂O (1 ml_) and vigorously stirred for 1 hour at 20 °C. The undissolved precipitate is collected by filtration, washed with H₂O (1 ml_) and dried in vacuum at 40 °C to give 143 mg (87 %) of rosuvastatin calcium salt (IX) as white powder.
1. A process for preparing a compound of formula IV or V where \( R_1 \) is a protecting group which comprises the following steps:
   a) converting a compound of formula I' where \( R_2 \) and \( R_3 \) are independently selected from d-C\(_4\) alkyl or together form a cyclic structure of formula \((\text{CH}_2)_n\) wherein \( n \) is from 2 to 6, into a compound of formula II where \( R_2 \) and \( R_3 \) are defined as above;
   b) subsequently converting said compound obtained in step (a) into a compound of formula III where \( R_1 \) is a protecting group, \( R_2 \) and \( R_3 \) are defined as above, and;
   c) subsequently cleaving the acetal-compound of formula III obtained in step (b).
2. The process according to claim 1 wherein R₂ and R₃ are methyl.

3. The process according to claim 1 or 2 wherein step (a) is performed with Br₂ and BaCO₃.

4. The process according to any of claims 1 to 3 wherein step (b) is performed by introducing R₁ being a silyl protecting group.

5. The process according to claim 4 wherein step (b) is performed with tert-butylmethylsilyl chloride.

6. The process according to any of claims 1 to 5 wherein step (c) is performed with I₂ and acetone by using Bronsted or Lewis acids in an organic solvent preferably Lewis acids such as FeCl₃·6H₂O, FeCl₃·SiO₂, CuCl₂·2H₂O, Zn(NO₃)₂·6H₂O, (NH₄)₂Ce(NO₃)₆, CeCl₃·7H₂O, TiCl₄, ZnBr₂, SnCl₂·2H₂O, LiBF₄ in wet acetonitrile, most preferably ZnBr₂ in methylene chloride; by transacetalization reaction with ketones in the presence of catalysts preferably with acetone in the presence of iodine; by pyridinium p-toluenesulfonate in wet acetone; DDQ; Montmorillonite K10; CBr₄/MeCN/H₂O; Me₃SiI.

7. The process according to any of claims 1 to 6 wherein step (c) is performed in an anhydrous medium to provide the compound of formula IV.

8. The process according to any of claims 1 to 6 wherein step (c) is performed in a solvent containing water to provide the hydrate compound of formula V.

9. A process for providing the compound of formula IV

\[
\text{IV}
\]

wherein R₁ is a protecting group, characterized by providing the compound of formula V
wherein \( R_1 \) is a protecting group,
dissolved in a solvent selected from the group of aromatic hydrocarbons, aliphatic hydrocarbons, chlorinated aromatic or aliphatic hydrocarbons and aliphatic ethers.

10. The process according to claim 9 wherein the solvent is selected from toluene, heptane, methylcyclohexane, cyclohexane, hexane, pentane, dichloromethane, chloroform, 2-chlorotoluene, 3-chlorotoluene, 4-chlorotoluene, \( t\text{BuMeO}, \text{Et}_2\text{O}, \text{Me}_2\text{O} \) and THF.

11. A compound of formula I

wherein \( R_1 \) is \( H \) or protecting group; and \( R_2 \) and \( R_3 \) are independently selected from \( C_1-C_4 \)-alkyl, or together form a cyclic structure of formula \((\text{CH}_2)_n\) wherein \( n \) is from 2 to 6.

12. The compound of claim 11, wherein \( R_1 \) is \( H \) or \( t\text{-butyldimethylsilyl} \).

13. The compound of claim 11 or 12, wherein \( R_1 \) and \( R_2 \) are methyl.

14. A process for preparing the lactol compound of formula I'

wherein \( R_2 \) and \( R_3 \) are independently selected from \( C_1-C_4 \)-alkyl, or together form a cyclic
structure of formula \((CH_2)_n\) wherein \(n\) is from 2 to 6, which comprises the step of reacting a substrate of formula \(X\)

\[
R_2\longrightarrow O\longrightarrow C\longrightarrow O\longrightarrow H\longrightarrow R_3
\]

\(X\)

5 wherein \(R_2\) and \(R_3\) are defined as above

with acetaldehyde under aldolase-catalysed aldol condensation conditions to form the corresponding lactol compound \(I'\).

15. The process according to claim 14 further comprising the additional steps of:
   a) converting said lactol compound of formula \(I'\) into a compound of formula \(IV\)

\[
\begin{align*}
O\longrightarrow C\longrightarrow O\longrightarrow &
\end{align*}
\]

10 \(IV\)

b) subjecting said compound obtained in step (a) to conditions sufficient to produce a HMG CoA reductase inhibitor or a pharmaceutically acceptable salt thereof.

16. The process according to claim 15 wherein the statin of step (b) is rosuvastatin.

17. The process according to any one of claims 14 to 16 wherein said substrate of formula \(X\) is selected from the group consisting of 2,2-dimethoxyethanal, 2,2-diethoxyethanal, 2,2-dipropoxyethanal, 2,2-dibutoxyethanal, 1,3-dioxolane-2-carbaldehyde, 4-methyl-1,3-dioxolane-2-carbaldehyde, 4-ethyl-1,3-dioxolane-2-carbaldehyde, 4-propyl-1,3-dioxolane-2-carbaldehyde, 4-butyl-1,3-dioxolane-2-carbaldehyde, 4,5-dimethyl-1,3-dioxolane-2-carbaldehyde.

18. The process according to any one of claims 14 to 17 wherein said aldolase is 2-deoxyribose-5-phosphate aldolase (DERA).

19. The process according to any one of claims 14 to 18 wherein said aldolase is DERA 01 comprising a nucleotide sequence of SEQ ID NO: 1 or an amino acid sequence
of SEQ ID NO: 2;
DERA 02 comprising a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 4 or an
amino acid sequence of SEQ ID NO: 5;
DERA 03 comprising a nucleotide sequence of SEQ ID NO: 6 or an amino acid sequence
of SEQ ID NO: 7;
DERA 04 comprising a nucleotide sequence of SEQ ID NO: 8 or an amino acid sequence
of SEQ ID NO: 9;
DERA 05 comprising a nucleotide sequence of SEQ ID NO: 10 or an amino acid
sequence of SEQ ID NO: 11;
DERA 06 comprising a nucleotide sequence of SEQ ID NO: 12 or an amino acid
sequence of SEQ ID NO: 13;
DERA 07 comprising a nucleotide sequence of SEQ ID NO: 14 or an amino acid
sequence of SEQ ID NO: 15;
DERA 08 comprising a nucleotide sequence of SEQ ID NO: 16 or an amino acid
sequence of SEQ ID NO: 17;
DERA 09 comprising a nucleotide sequence of SEQ ID NO: 18 or an amino acid
sequence of SEQ ID NO: 19;
DERA 10 comprising a nucleotide sequence of SEQ ID NO: 20 or an amino acid
sequence of SEQ ID NO: 21;
DERA 11 comprising a nucleotide sequence of SEQ ID NO: 22 or an amino acid
sequence of SEQ ID NO: 23;
DERA 12 comprising a nucleotide sequence of SEQ ID NO: 24 or an amino acid
sequence of SEQ ID NO: 25;
DERA 13 comprising a nucleotide sequence of SEQ ID NO: 26 or an amino acid
sequence of SEQ ID NO: 27;
DERA 14 comprising a nucleotide sequence of SEQ ID NO: 28 or an amino acid
sequence of SEQ ID NO: 29;
DERA 15 comprising a nucleotide sequence of SEQ ID NO: 30 or an amino acid
sequence of SEQ ID NO: 31;
DERA 16 comprising a nucleotide sequence of SEQ ID NO: 32 or an amino acid
sequence of SEQ ID NO: 33;
DERA 17 comprising a nucleotide sequence of SEQ ID NO: 34 or an amino acid
sequence of SEQ ID NO: 35;
DERA 18 is an aldolase comprising a nucleotide sequence of SEQ ID NO: 36 or an amino
acid sequence of SEQ ID NO: 37;
DERA 19 comprising a nucleotide sequence of SEQ ID NO: 38 or an amino acid
sequence of SEQ ID NO: 39;
DERA 20 comprising a nucleotide sequence of SEQ ID NO: 40 or an amino acid sequence of SEQ ID NO: 41;
DERA 21 comprising a nucleotide sequence of SEQ ID NO: 42 or an amino acid sequence of SEQ ID NO: 43;
DERA 22 comprising a nucleotide sequence of SEQ ID NO: 44 or an amino acid sequence of SEQ ID NO: 45;
DERA 23 comprising a nucleotide sequence of SEQ ID NO: 46 or an amino acid sequence of SEQ ID NO: 47;
or an aldolase having an amino acid sequence identity of at least about 70% to amino acid sequence of any of said aldolases

20. The process according to claim 19 wherein said aldolase has an amino acid sequence identity of at least about 70% to amino acid sequence of SEQ ID NO: 2.

21. The process according to claim 19 wherein said aldolase has an amino acid sequence identity of at least 90% to amino acid sequence of SEQ ID NO: 5.

22. The process according to claim 19 wherein said aldolase has an amino acid sequence identity of at least 90% to amino acid sequence of SEQ ID NO: 17.

23. The process according to claims 14 to 22 wherein a buffer having no primary, secondary or tertiary amino group is used.

24. The process according to claim 23 wherein said buffer is a phosphate buffer.

25. The process according to any one of claims 14 to 24 wherein pH value for the aldolase-catalysed aldol condensation is maintained in range 6 to 10.

26. The process according to claim 25 wherein pH is maintained with a buffer in the range of 7 to 9.

27. The process according to any one of claims 14 to 24 comprising the steps of
   i) performing the aldolase-catalysed aldol condensation in an aqueous medium at pH value of 7 to 9 at a temperature of 30 - 50°C to form a reaction mixture,
   ii) maintaining said reaction mixture at said temperature and pH value for a time period between 1 h and 6 h; and
   iii) recovering said compound of formula I'.
28. A use of aldolase for the reaction of a substrate of the formula X

\[ \begin{align*}
R_2 & \quad O \\
H & \\
R_3 & \quad O
\end{align*} \]

wherein \( R_2 \) and \( R_3 \) are independently selected from \( \text{Ci-C}_4 \)-alkyl, or together form a cyclic structure of formula \((\text{CH}_2)_n\) wherein \( n \) is from 2 to 6,

with acetaldehyde under aldolase-catalysed aldol condensation conditions to form the corresponding lactol compound of the formula I'

\[ \begin{align*}
\text{HO} & (R) \\
\text{OH} & (S) \\
\text{R}_2 & \\
\text{OR}_3
\end{align*} \]

wherein \( R_2 \) and \( R_3 \), wherein \( R_2 \) and \( R_3 \) are defined as above.

29. The use according to claim 28 wherein said aldolase is 2-deoxyribose-5-phosphate aldolase (DERA).

30. The use according to claim 28 or 29 wherein aldolase is

DERA 01 comprising a nucleotide sequence of SEQ ID NO: 1 or an amino acid sequence of SEQ ID NO: 2;

DERA 02 comprising a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 4 or an amino acid sequence of SEQ ID NO: 5;

DERA 03 comprising a nucleotide sequence of SEQ ID NO: 6 or an amino acid sequence of SEQ ID NO: 7;

DERA 04 comprising a nucleotide sequence of SEQ ID NO: 8 or an amino acid sequence of SEQ ID NO: 9;

DERA 05 comprising a nucleotide sequence of SEQ ID NO: 10 or an amino acid sequence of SEQ ID NO: 11;

DERA 06 comprising a nucleotide sequence of SEQ ID NO: 12 or an amino acid sequence of SEQ ID NO: 13;

DERA 07 comprising a nucleotide sequence of SEQ ID NO: 14 or an amino acid sequence of SEQ ID NO: 15;

DERA 08 comprising a nucleotide sequence of SEQ ID NO: 16 or an amino acid sequence of SEQ ID NO: 17.
sequence of SEQ ID NO: 15;
DERA 08 comprising a nucleotide sequence of SEQ ID NO: 16 or an amino acid sequence of SEQ ID NO: 17;
DERA 09 comprising a nucleotide sequence of SEQ ID NO: 18 or an amino acid sequence of SEQ ID NO: 19;
DERA 10 comprising a nucleotide sequence of SEQ ID NO: 20 or an amino acid sequence of SEQ ID NO: 21;
DERA 11 comprising a nucleotide sequence of SEQ ID NO: 22 or an amino acid sequence of SEQ ID NO: 23;
DERA 12 comprising a nucleotide sequence of SEQ ID NO: 24 or an amino acid sequence of SEQ ID NO: 25;
DERA 13 comprising a nucleotide sequence of SEQ ID NO: 26 or an amino acid sequence of SEQ ID NO: 27;
DERA 14 comprising a nucleotide sequence of SEQ ID NO: 28 or an amino acid sequence of SEQ ID NO: 29;
DERA 15 comprising a nucleotide sequence of SEQ ID NO: 30 or an amino acid sequence of SEQ ID NO: 31;
DERA 16 comprising a nucleotide sequence of SEQ ID NO: 32 or an amino acid sequence of SEQ ID NO: 33;
DERA 17 comprising a nucleotide sequence of SEQ ID NO: 34 or an amino acid sequence of SEQ ID NO: 35;
DERA 18 is an aldolase comprising a nucleotide sequence of SEQ ID NO: 36 or an amino acid sequence of SEQ ID NO: 37;
DERA 19 comprising a nucleotide sequence of SEQ ID NO: 38 or an amino acid sequence of SEQ ID NO: 39;
DERA 20 comprising a nucleotide sequence of SEQ ID NO: 40 or an amino acid sequence of SEQ ID NO: 41;
DERA 21 comprising a nucleotide sequence of SEQ ID NO: 42 or an amino acid sequence of SEQ ID NO: 43;
DERA 22 comprising a nucleotide sequence of SEQ ID NO: 44 or an amino acid sequence of SEQ ID NO: 45;
DERA 23 comprising a nucleotide sequence of SEQ ID NO: 46 or an amino acid sequence of SEQ ID NO: 47.

The use according to any of claims 28 to 30 wherein said aldolase is comprised within a living whole cell, or is comprised within an inactivated whole cell, or is comprised within a homogenized whole cell, or is comprised within a cell free extract, or is a purified enzyme,
or is immobilized, or is in form of an extracellularly expressed protein.

32. A process for preparing a HMG CoA reductase inhibitor characterized in that the compound of formula I' is provided as an intermediate compound to prepare said HMG CoA reductase inhibitor.

\[
\begin{align*}
\text{I'} & \quad \text{R}_2 \text{O} \quad \text{OR}_3 \\
\end{align*}
\]

wherein \( R_2 \) and \( R_3 \) are independently selected from \( d\)-C-alkyl or together form a cyclic structure of formula \((\text{CH}_2)_n\) wherein \( n \) is from 2 to 6,

33. The process according to claim 32 comprising the following steps:
   i) converting a compound I' into a compound of formula IV

\[
\begin{align*}
\text{IV} & \quad \text{R}_1 \text{O} \\
\end{align*}
\]

wherein \( R_1 \) is a protecting group; and

ii) reacting said compound of formula IV under the conditions sufficient to produce a HMG CoA reductase inhibitor or a pharmaceutically acceptable derivative thereof.

34. The process according to claim 33 wherein the conditions of step (II) are set by a Wittig coupling with an appropriate phosphonium salt to give rosuvastatin or a derivative thereof, preferably wherein the process comprises the steps of:

\[
\begin{align*}
\text{VI} & \quad \text{Y} \\
\end{align*}
\]

Na) providing a phosphonium salt having the formula VI.
wherein Rx, Ry, and Rz, are the same or different and are selected from optionally substituted d-Os-alkyl or C_3-C_6-cycloalkyl or d-Cs-alkenyl or C_5-C_6-cycloalkenyl or aryl, and Y is an anion, preferably halogen or RCOO anion, more preferably chloride, bromide or trifluoroacetate;

to give a compound of formula VII

![Chemical structure](image)

and

lib) subsequently converting compound VII to rosuvastatin or a salt thereof.

35. The process according to any of claims 32 to 34 wherein R_2 and R_3 are methyl.

36. A process for preparing a HMG CoA reductase inhibitor wherein the compound of formula II

![Chemical structure](image)

wherein R_2 and R_3 are independently selected from d-C_4-alkyl or together form a cyclic structure of formula (CH_2)_n wherein n is from 2 to 6, is provided as an intermediate compound to prepare said HMG CoA reductase inhibitor, which process comprises the steps of:

A) converting a compound II into a compound of the formula IV

![Chemical structure](image)
wherein $R_1$ is a protecting group; and

B) reacting said compound of formula IV by a Wittig coupling with an appropriate phosphonium salt to give a HMG CoA reductase inhibitor.

37. The process according to claim 36 wherein the HMG CoA reductase inhibitor is rosuvastatin or a derivative thereof.

38. The process according to claim 36 or 37, wherein $R_2$ and $R_3$ are methyl.